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16. ABSTRACT
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DNA Adsorption to Soils and Sediments

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■ Deoxyribonucleic acid (DNA) adsorption to five soils, an acid-washed sand, and a lake sediment was investigated. All DNA at environmentally relevant concentrations was adsorbed by soils containing a significant amount of montmorillonite at low to neutral pH values. Studies on the effects of DNA molecular size on adsorption to sand and a sandy soil were described by the Freundlich isotherm model ($r^2 > 0.85$) and indicated a direct relationship between molecular weight and adsorption.

Introduction

The recovery of deoxyribonucleic acid (DNA) from environmental samples as a measure of microbial biomass and activity, uptake, excretion, and turnover of nucleotides and nucleic acids (*1*) and the impact and transfer of recombinant DNA in the environment (*2*) are the focus of considerable interest. DNA recovery from environmental matrices (*3–6*) has been demonstrated. This DNA may be extracellular DNA excreted or released by death and cell lysis, or cellular DNA released by lysis procedures used for DNA recovery. Recent research has demonstrated that quantitative detection of specific DNA sequences in environmental DNA extracts is feasible (*2, 7*) and that new direct environmental approaches for measuring microbial genetic diversity are possible (*7*).

A primary factor controlling the fate of DNA in the environment and the efficiency of DNA recovery from environmental samples is the extent and mechanism by which DNA is sorbed to particulates, soils, and sediments. Sorption may also determine the availability of free DNA for attack by extracellular DNase and the uptake by bacteria as a nutrient or for incorporation into genomes (*8, 9*).

We have analyzed DNA adsorption isotherms for a variety of soils and model sediments that represent a range of clay and organic matter contents. The effects of DNA polymer size have also been investigated.

Experimental Section

Five different soils, a lake sediment, and an acid-washed sand were used in this study, and the pertinent properties of these materials are listed in Table I. All materials were ground to pass through an 80-mesh sieve (nominal diameter, 177 μm).

Commercially obtained herring sperm DNA (Sigma, St. Louis) was used in all experiments. For the high molecular weight DNA studies, the DNA was dissolved in standard saline citrate (SSC) (0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) after several passes through a 21-gauge needle. DNA for the low molecular weight DNA sorption isotherms was sheared by four passes through a French pressure cell press (Aminco Inc.) at 20 000 psi. The fragment size of this DNA was found to average 500 base pairs by agarose gel electrophoresis with λ-phage DNA restricted by *Hin*dIII as a size marker (data not shown).

The extent of DNA sorption to these soils was determined by a batch slurry method similar to that described by Green et al. (*10*). All glassware was siliconized by treatment with dimethyl dichlorosilane to prevent DNA adsorption to glass. Two grams of soil was weighed into siliconized glass tubes, SSC added to soil to 1% on the basis of mass of soil, and autoclaved for 30 min to inactivate heat-labile nucleases. Four milliliters of DNA solution (concentrations ranging from 0 to 100 μg/mL) was added to the soils with three replicates at each concentration, mixed well, and shaken on a tumbling shaker overnight. These tubes were centrifuged at a low speed until the supernatant was clear, and the supernatant was analyzed for DNA concentration as described below. The amount of DNA removed from solution was calculated as DNA sorbed by the soil or sediment. The sorption data was analyzed by the Freundlich equation

$$S = KC_e^n$$

where S is the sorbed concentration (μg/g), C_e is the solution concentration (μg/mL), and K and n are constants, or by the Langmuir equation

$$S = S_{max}KC_e/(1 + KC_e)$$

where S_{max} is the maximum adsorption capacity (μg/g), and S and C_e are as defined for the Freundlich case (*11*). K is defined as the Langmuir affinity coefficient (mL/μg). pH was determined by using the appropriate buffer in a 2:1 solution:soil mixture after being stirred for 1 h.

DNA concentrations were analyzed in one of two ways. Concentrations of high molecular weight DNA were determined by a fluorometric method similar to that described by Paul and Myers (*12*). Two milliliters of supernatant was mixed with 1 mL of 1.5×10^{-5} M Hoeshct 33258 fluorescent dye (Sigma) and allowed to sit in the dark for 10 min, and the concentrations were analyzed by a Perkin-Elmer Model MPF-44A fluorescent spectrophotometer set at an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Standard curves were prepared with a range of DNA concentrations in the appropriate soil extract. The DNA concentrations for the molecular weight studies were determined by using radio-labeled DNA. DNA solutions were spiked to 20 000 cpm/mL with ^{32}P nick translation labeled (*13*) sheared herring sperm DNA before addition to soil, and the equilibrium concentrations were related to radioactivity remaining in solution, determined by the addition of 0.5 mL of supernatant to 10 mL of commercial scintillation cocktail (Beckman Ready-Solv HP/b), and liquid scin-

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Table I. Selected Properties of Soils and Sediments

soil	texture	minerals	clay	organic carbon, %	CEC,[b] mequiv/100 g
Maury	silt loam	vermiculite, montmorillonite, kaolinite, illite, quartz	24.0	0.90	17.50
Iberia	silt clay	montmorillonite, kaolinite	49.3	2.16	40.80
Memphis	silt	montmorillonite, illite	10.0	0.76	6.50
Lily	loam	vermiculite, illite	14.0	1.98	4.85
Eustis	fine sand	quartz, montmorillonite	3.2	0.60	5.20
acid-washed sand	sand	quartz	0	0	ND[a]
Ft. Loudon sediment	silt	interstratified material, kaolinite, montmorillonite	26.0	3	ND[a]

[a] ND = not determined. [b] CEC = cation-exchange capacity.

Table II. DNA Sorption Model Characteristics to Soils and Sediments

Freundlich Fit

soil/ sediment	DNA, molecular weight	pH	K (μg/g)-(μg/mL)$^{-n}$	n	r^2
Eustis	high	6.8	2.36	0.95	0.87
	low	6.8	1.51	0.86	0.95
acid-washed	high	6.8	2.82	0.68	0.90
sand	low	6.8	3.15	0.42	0.99

Langmuir Fit

soil	DNA molecular weight	pH	K (μg/g)	r^2
Memphis	high	7.2	1.02	0.95

[a] All DNA added to Maury (pH 6.1), Iberia (pH 6.3), Ft. Loudon (pH 6.5), and Memphis (pH 5.8) was adsorbed. No DNA added to Lily (pH 6.9) or to Memphis (pH 10) was adsorbed.

tillation spectrometry (Tracor Analytic Model 6892).

The range of DNA concentrations used in this study (10–200 μg/g soil) is environmentally relevant on the basis of previous studies. Maeda and Taga (*15*) reported total DNA concentrations of up to 35 μg/g of ocean sediment, and Torsvik and Goksoyr (*16*) found up to 90 μg of bacterial DNA per g of dry soil.

Results and Discussion

As summarized by Table II, DNA sorption to Eustis soil and acid-washed sand was described by the Freundlich model. DNA sorption to Memphis soils was described by the Langmuir model, and for Maury and Iberia soils and the Ft. Loudon sediment, all DNA added (up to 200 μg/g) was sorbed, and consequently neither model was fit. No DNA was sorbed by the Lily soil.

Adsorption of DNA by Eustis soils (a sandy soil) and acid-washed sand was studied at two different shear sizes (Figure 1). In both sands unsheared DNA was sorbed to a greater extent than the sheared DNA, as well as showing a more linear isotherm ($n \rightarrow 1$) in the range of concentrations studied.

The adsorption isotherm for acid-washed sand is somewhat different from the Eustis soils in that the K's for the unsheared DNA versus the sheared DNA are very close. The sheared DNA isotherm resembles that of the unsheared DNA at low concentrations, but more sheared DNA is adsorbed at equilibrium concentrations greater than 20 μg/mL. The Eustis isotherms are much more linear than those for acid-washed sand, indicating a higher sorptive capacity. This may be due to the presence of clay minerals, such as small amounts of smectites (Table I) in the Eustis soil that were not present in the sand. Divalent cations may also be present in Eustis, which may have participated in cation bridging to the surface of the soil

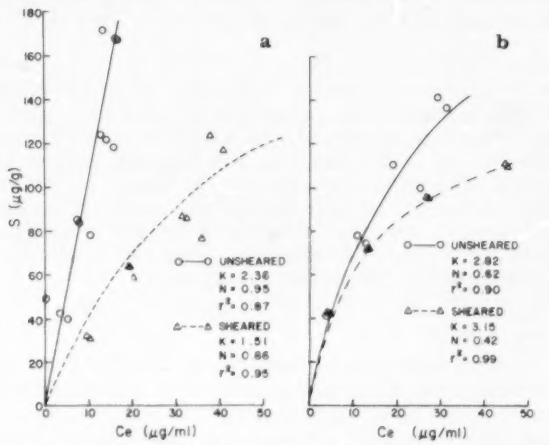

Figure 1. Sorption of high (unsheared) and low (sheared) molecular weight DNA to Eustis soil and acid-washed sand. Both sets of data were analyzed by the Freundlich model. (a) Eustis soil and (b) acid-washed sand.

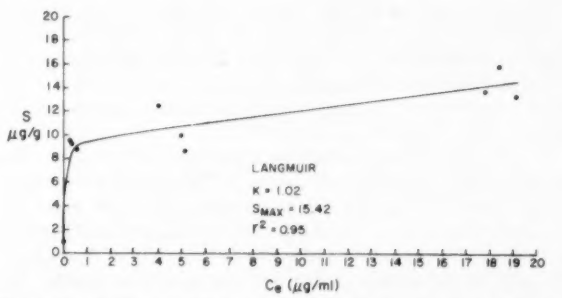

Figure 2. Memphis soil DNA sorption isotherm at pH 7.2. This soil showed a saturation Langmuir type isotherm even in the presence of a significant amount of montmorillonite.

similar to the effect observed for sand (*14*).

Previous reports indicate that montmorillonite clay dominates the sorption of DNA at low to neutral pH values. Greaves and Wilson (*15*) have reported that pure montmorillonite may adsorb more DNA than its own weight at pH 5, although this is unlikely for a natural clay due to adsorbed organic matter. The intercalation of DNA in montmorillonite appears to be dependent upon the ionic state of the DNA. At low pH values (below 7 in these soils), DNA is neutral and moves into the interlamellar space where it is adsorbed. At higher pH values (DNA has a pK_a of approximately 5), DNA is in the ionic form and is excluded from intercalation (*9*). The pH of the microenvironment surrounding a soil particle may be as much as 2 or 3 pH units below the pH of the bulk solution. As can be seen from Table II, soils and sediments that contain a significant amount of montmorillonite adsorb all of the DNA added to the system. The exception is the Memphis

soil (Figure 2), which contains approximately 3% montmorillonite and adsorbs a maximum of 15.4 μg/g DNA, described by the Langmuir equation (S_{max} = 15.4 μg/g). The pH of this soil in SSC is relatively high (7.2), and it is likely that not all of the DNA was neutral (the molecular form). That DNA which was neutral may have moved between the clay layers and was adsorbed, whereas the remaining ionized DNA was not sorbed. Further, when the pH of the solution was adjusted to pH 10 with NaOH, no DNA was sorbed, and when the pH was adjusted to pH 5.8 with HCl, all of the DNA was sorbed. This indicates that the surface pH of some natural soils and sediments may be near the pK_a of DNA and that significant amounts of DNA could be present in the aqueous phase even in the presence of montmorillonite.

The contribution of organic carbon to DNA sorption appears to be minimal at the pH values of the soils studied. Lily soil, which does not contain montmorillonite and 1.98% organic carbon (Table I), adsorbed no DNA, while Iberia, which contained a similar amount of organic carbon (2.16%) and a significant amount of montmorillonite, adsorbed all DNA added.

The adsorption of DNA to sediments and soils is affected by a number of factors, including the mineralogy of the sorbent, the ionic strength and pH of the medium, and the length of the DNA polymer. Knowledge and control of these factors are required to accurately measure DNA concentrations and fate in the environment and in the further development of protocols for the efficient extraction and purification of DNA from natural microbial communities.

Registry No. Montmorillonite, 1318-93-0.

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